Downstream Processing
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Lecture - 06
Cell Breakage

Today we are going to talk about cell breakage. It could be a bacterial or a fungal, animal or a plant cell.

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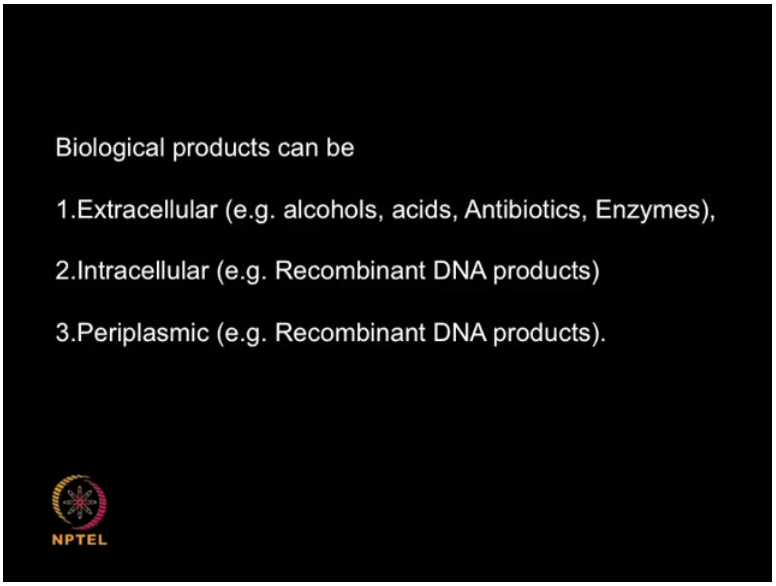

What happens is if your product of interest is held up inside the microorganism, then you need to release those products and then perform your purification task. So, generally biological products could be extracellular like alcohols, acids, antibiotics and some enzymes. It could be intracellular; sometimes you have recombinant DNA products.

It could be periplasmic. That means it could be in between also. So, you can have different types of products being produced and depending upon where the product is getting accumulated, you may have to resort to breaking the cells and releasing the contents. Sometimes, the release of the product from intracellular to extracellular may be diffusion controlled and it may take a very long time for you to get all the products out. So, in such a situation also, you may resort to cell breakage.

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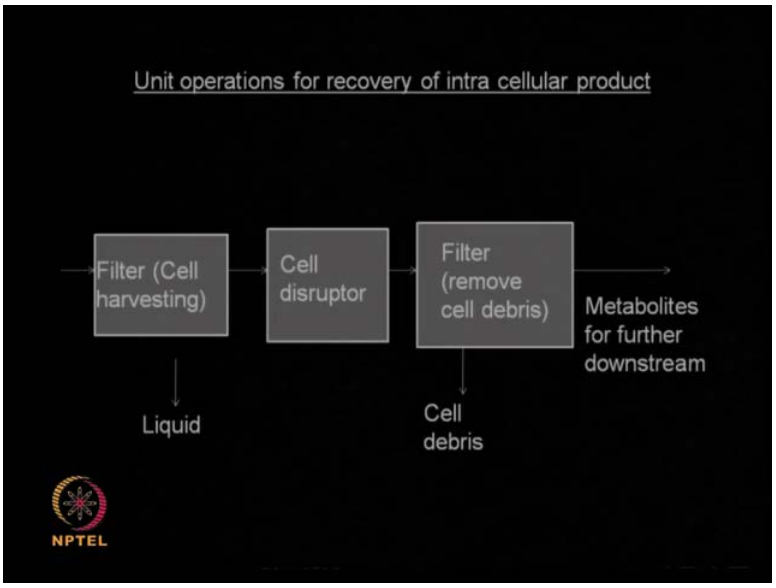

So, if you want to perform a cell breakage type of operation that means if you are interested in recovering an intracellular product, then you have extra downstream operations to perform. So, you need to collect all the cells that is called cell harvesting and then you need to resort to a cell disruption. This could be a mechanical, it could be a chemical, it could be enzymatic, and it could be a physical operation. We are going to spend more time on this particular box, the second box. Then, once you have released the product from the cell, you need to filter the cell debris, the dead biomass.

Then, the liquid is going to contain your product of interest, the metabolite or it could be a protein or it could be a small molecule. So, here you are having cell debris that is being removed, whereas here, you are interested in the cells. I had talked, mentioned before that if it is an extracellular product; you are not interested in the cells.

So, cells are removed in the very first stage of filtration, but if it is an intracellular product, the cells are harvested. Then, you break the cells and then you collect the liquid part of it, which will contain hopefully all your product. The dead cells, the broken cells are thrown out. So, there will be extra downstream operations if you are interested in the intracellular product.

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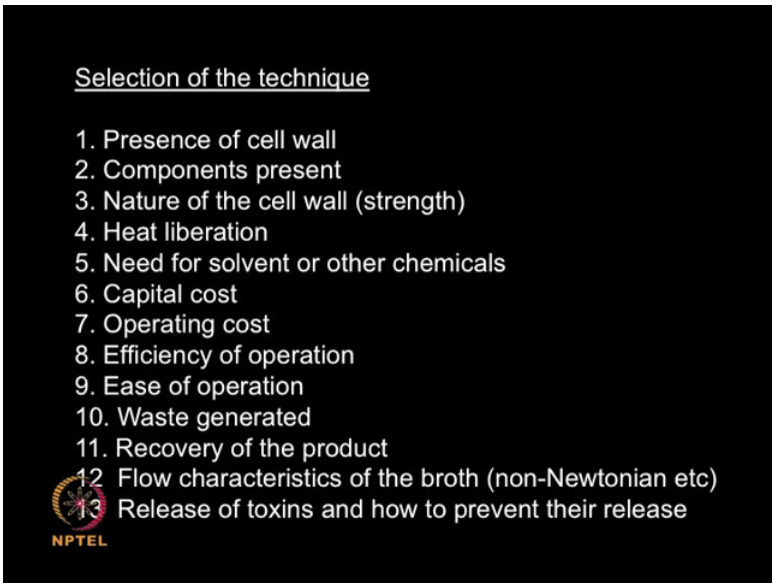

There are many points you need to consider when you are going to select a technique for disrupting the cells. As I mentioned, it could be a physical method, it could be a chemical method, it could be an enzymatic method, but you need to consider several points before you decide, which technique I am going to follow. Does the microorganism have a cell wall? What are the components present in the cell wall?

Third is the nature of the cell wall. Is it very strong, tough or is it very fragile? Can I very easily break the cell wall or I need to resort to some extra mechanical techniques to break the cell wall? So, depending upon the strength, I may select my technique. Heat liberation, am I going to generate lot of heat? If I my going to generate heat, as you know enzymes are very susceptible to thermal gradients. So, they may lose their activity. So, am I going to generate lot of heat? Then, may be that method is not good, I have to go to some other method.

Do I need lot of solvents or any other chemicals? For example, enzymatic methods or chemical methods will require some extra solvents or chemicals or solubilizers. So, do I require all these? What is the capital cost? That means what type of equipment I am going to put in? What will be the equipment cost? That is called capital cost, and then comes operating cost. How much energy I will require to do this job? Do I require cooling water? Do I require inert conditions? Do I require chilling water? So, that is the operating cost and the efficiency of the process. How efficient is the process?

Ideally, if you are interested in the intracellular product, you would like to collect 100 percent of the intracellular material. So, you would like to have 100 percent efficient process, but then that is not so in reality. You may have different amount of efficiency vary from 30, 40, 50, going up to 70, 80. So, you would like to know the efficiency of the process. Sometimes, you may have to redo it, second time, third time that is called passes. So, you may have a second pass, third pass; fourth pass until you recover all your product.

So, you need to know the efficiency of the process. If you are going to do it many passes obviously, the operating cost is very high. So, that is not very favorites. So, if the efficiency is very high, then you will not have many passes. Ease of operation is how easy it is to operate I am going to talk about bead mill, where the operation is problematic. You need to after the performance; you need to wash the setup. You need to remove the beads and clean each one of them. So, the ease of operation is very important.

Once I have done the cell breakage, can I use the set up for the next batch and the third batch and so on? What is the waste generated? That means how much waste apart from the cell debris, the dead biomass, the intracellular material, may be EPS, DNA. How much of waste am I generating? Am I going to generate lot of waste? Recovery of the product, once I have done the cell breakage, how easy it is to recover my product because I am going to have lot of solid material, lot of viscous material. My product of interest is also going to part of it. Is it going to be very easy to recover?

So, I need to consider that aspect as well. Then, flow characteristics of the broth, once you have broken down the cells, the whole solution becomes non Newtonian. As you know, non Newtonian fluids behave very differently unlike Newtonian fluid. So, when you apply more shear, the viscosity changes in a non linear fashion. So, when you have a non Newtonian fluid, it becomes very difficult for you to do the mixing, pumping, handling, flow and so on actually. So, am I generating lot of non Newtonian fluid behavior? Once I have broken down the cells, you need to consider that aspect as well. Then finally, am I releasing toxins?

When I do a cell breakage, I am not only releasing product. But, am I releasing inhibitors, which are going to deactivate my protein or enzyme? Am I going to release

toxins, which are harmful for the product of interest? So, you need to consider that also. There are a large number of criteria, which you need to consider before you decide on which type of cell breakage equipment you are going to resort to. So, all these are very important. All these affect your overall plan and overall efficiency of the entire downstream operation. We will talk about some of them in the next course of this lecture as well as possibly the next lecture as well, but not all the points.

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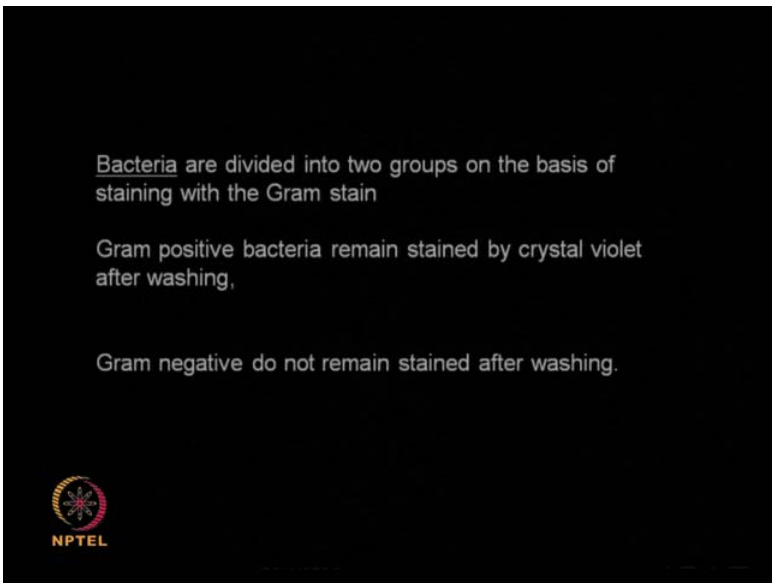

Let us look at some microorganisms. You might have done courses on microbiology. So, you already may know the structure of the microorganism and what it contains and so on. But, it is very important in this particular context to know that as well because as I mentioned in the previous slide, you must know what is the composition of the cell, how tough is the cell wall, what is the ease of breaking of the cell wall. When you break the microorganism, am I going to generate inhibitor or toxin?

So, in order to know all these, you need to understand certain details about the cell walls of bacteria, cell walls of yeast, animal cells, and plant cells and so on. That will give you an idea in selecting the correct equipment. Let us start from bacteria. You may know about this in your microbiology class. As I mentioned before, so there are two types of bacteria, the gram positive and the gram negative.

So, it depends based on the staining. So, gram positive remains stained by crystal violet even after washing. Gram negative do not remain stained after washing. That is how

bacteria have been classified into two broad categories. Why does the stain remain, why does the stain get removed that depends because of the presence of the cell wall. We will talk about it as we go along.

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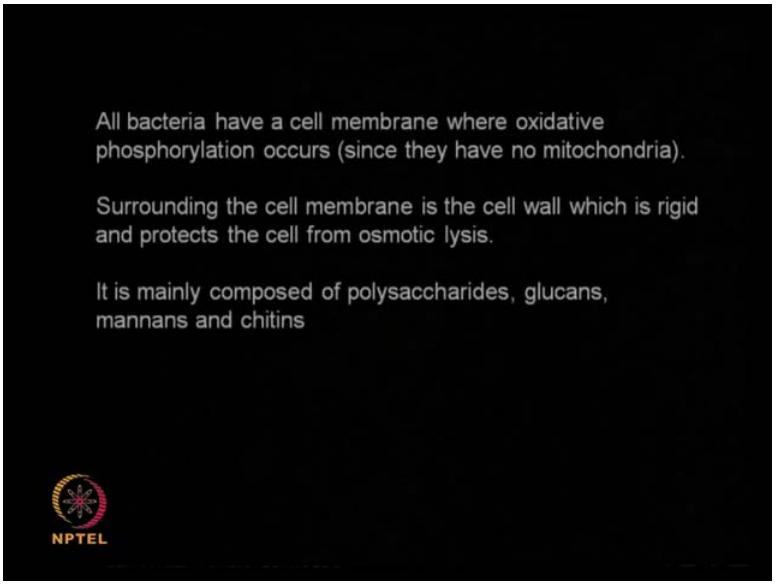

So, they all have a cell membrane where oxidative phosphorylation takes place. They do not have mitochondria surrounding the cell membrane. There is a cell wall which is rigid and protects the cell from osmotic lysis. So, the cell wall's job is to protect. What does the cell wall contain? It contains glucans, it contains mannans and chitins. So, as we can see glucans, many types of glucans may be present, many types of polysaccharides may be present. So, it is a mixture of different molecular weights of these products. So, that is what is the cell wall is going to contain in a bacteria.

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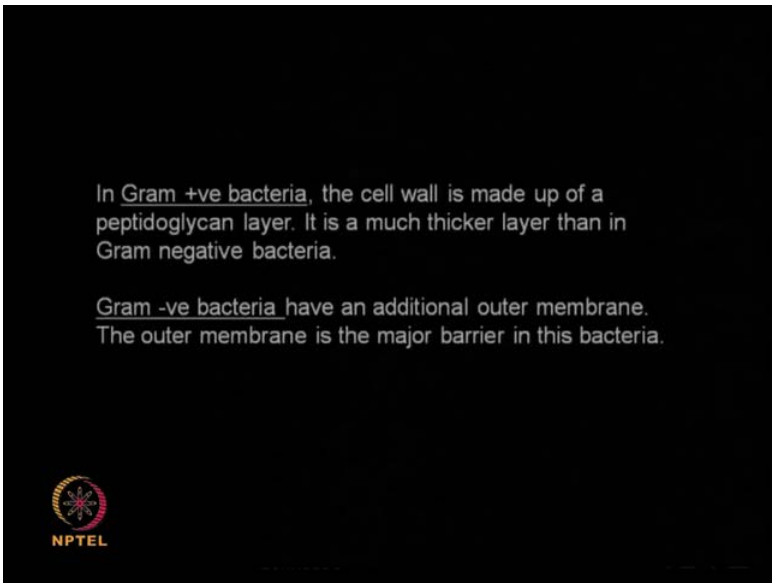

The gram positive cell wall is made up of a peptidoglycan layer. It is a thick layer when compared to the gram negative, whereas gram negative has an additional outer membrane. This outer membrane is the major barrier in these bacteria. So, there is a difference between gram positive and gram negative bacteria. That difference you need to consider if you are going to select your breakage equipment, whether my organism is a gram positive or a gram negative.

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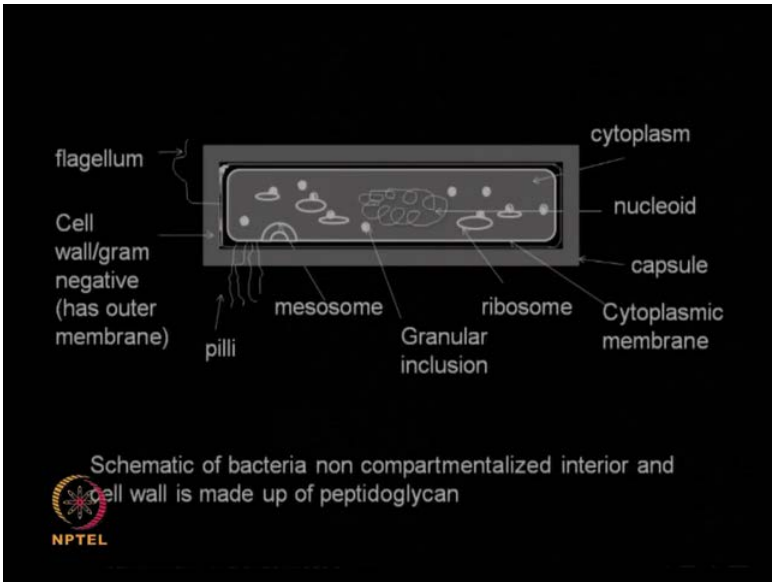

This is just a schematic of how a bacteria looks like. So, it is a non compartmentalized

interior cell wall and it is made up of peptidoglycan. So, it has got a cell wall. So, gram negative has an outer membrane here. Then, you have the cytoplasm. Then, you have the nuclei. Then, we have the capsule is the cytoplasmic membrane here. Then, you have the ribosome; granular inclusions are present here, flagellum, pilli and so on. So, this is a typical schematic of bacteria. Now, let us look at difference between a gram positive and the gram negative bacteria.

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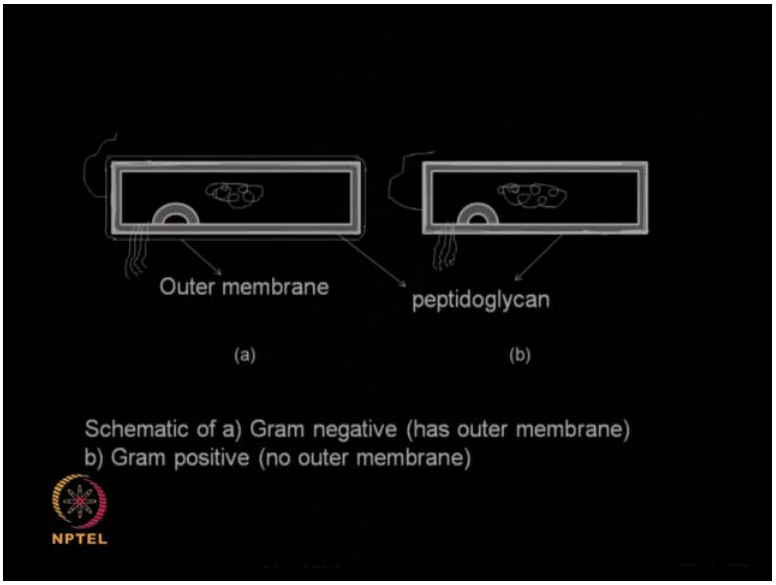

So, you have both of them have this peptidogly can, but the gram negative has an outer membrane here, whereas, a gram positive does not have an outer membrane. So, that is the difference between both these bacteria.

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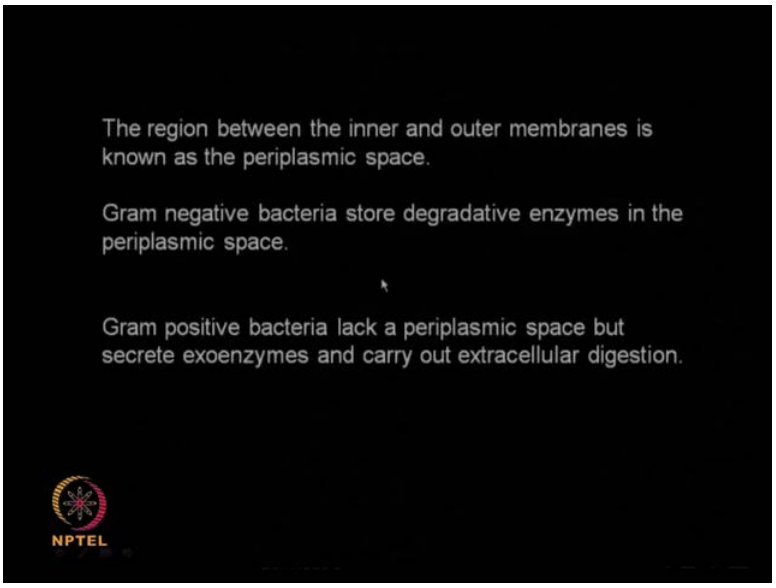

The region between the inner and the outer membranes, this is known as the periplasmic space. Sometimes, your product of interest may be found in the periplasmic space. It is not exactly in the interior of the microorganism that means intracellular, but it is also not in the extracellular. But, it might be in the periplasmic space. So, but still, you need to break the microorganism. But, you do not have to break it too much because it is not inside the microorganism unlike the intracellular material. So, you can resort to slightly milder cell breakage techniques if your product is in the periplasmic region. Understand?

If the product is inside intracellular, then you need to really break it very hard. Gram negative bacteria store degradative enzymes in the periplasmic space because they do quite a lot of degradation of the various carbon sources extracellularly. So, they store the degradative enzymes, which get released in the extracellular space for degrading its carbon source. Gram positive bacteria lack this periplasmic space, but they also secrete certain exoenzymes and it carries out the extracellular digestion actually. So, gram negative has degradative enzymes in the periplasmic space, whereas this one secretes exoenzymes and carries out the extracellular digestion.

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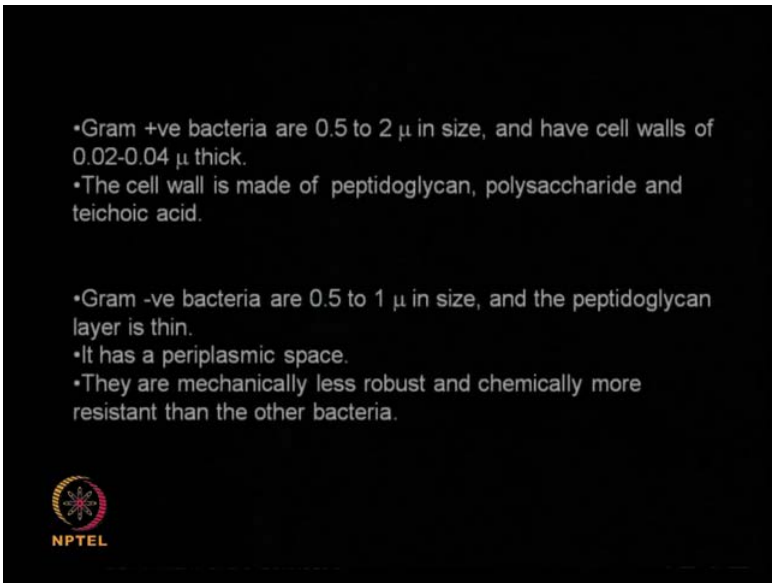

Let us look at sizes of these microorganisms. It is very important to know because I can select my equipment based on the size. So, gram positive bacteria are about 0.5 to 2 micron in size and they have cell walls of about 0.02 to 0.04 micron thick. The cell wall is made up of as I mentioned the peptidoglycan, polysaccharide and teichoic acid. Now, if you look at gram negative, it is about 0.5 to 1 micron in size.

This peptidoglycan layer is very thin. This also has a periplasmic space. These are less, mechanically less robust. That means if I use the mechanical method, I can easily break gram negative bacteria when compared to gram positive bacteria, but they are chemically more resistant than gram positive.

So, if I want to resort to chemical methods, then gram positive bacteria are more susceptible than gram negative bacteria. Do you understand? So, if I want to resort to mechanical methods, gram negative bacteria are less robust. So, they can easily broken down when compared to gram positive. If I want to use a chemical method, then gram positive is more susceptible than gram negative bacteria. So, Ii can select either the mechanical or the chemical method depending upon whether it is a gram positive or gram negative bacteria.

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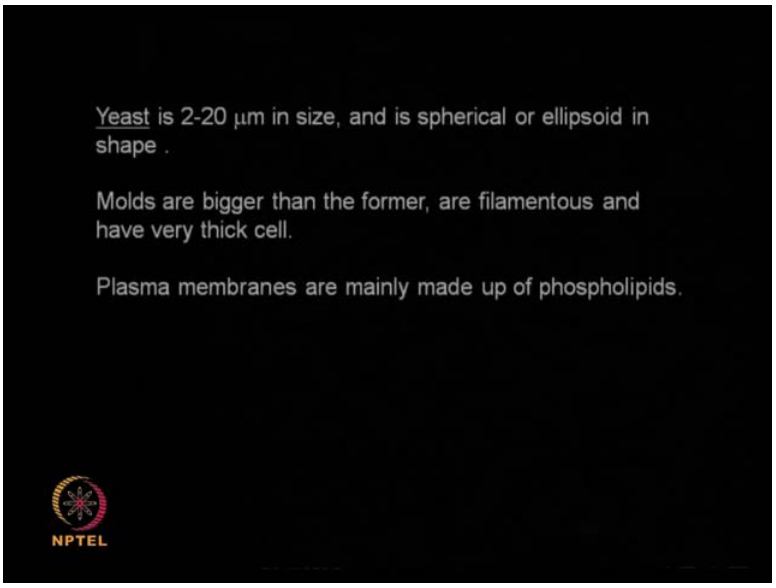

Now, let us look at yeast. Yeast is slightly bigger, 2 to 20 micron in size. They are generally spherical in shape or sometimes ellipsoidal. They are quite tough when compared to bacteria. Then, we have things like molds. They are very big. They are filamentous. You can have molds, filamentous mold and they can have a very thick wall. The plasma membranes are mainly made up of phospholipids.

So, if you want to break yeast or molds, you need to resort to much higher shear stresses when compared to the bacteria and molds if you are going to have filamentous material also present, it may get entangled in certain mechanical agitators. So, that is a very important point you need to consider.

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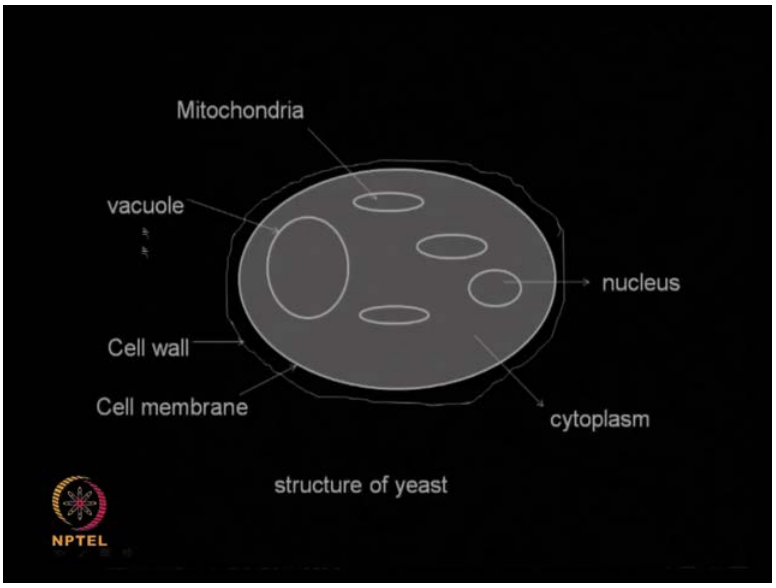

This is a typical schematic of yeast. So, you have mitochondria here. This is the cell wall as you can see. Then, you have the cell membrane. We have the cytoplasm. Then, you have the nucleus here. As I said, the yeast are much bigger than the gram positive or the gram negative bacteria.

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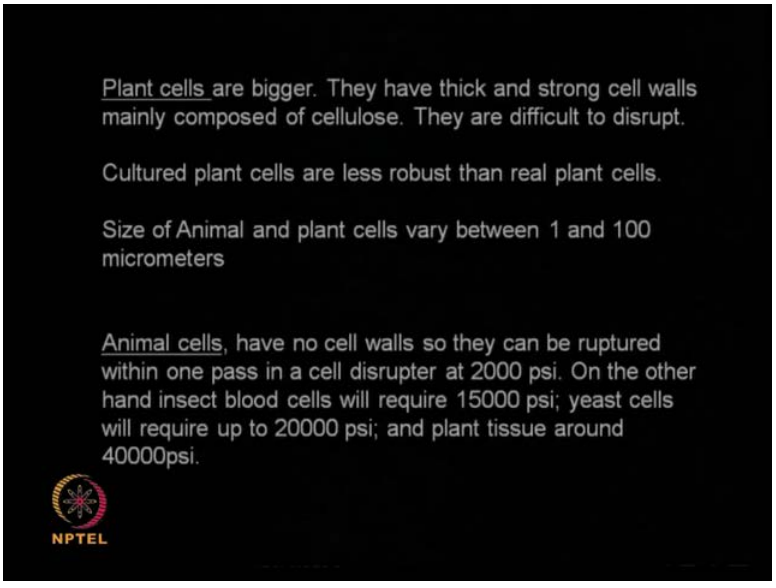

Now, let us look at plant cells. They are quite big. They have thick and strong cell walls composed of cellulose. So, because cellulose is very tough, breaking plant cells, is a big challenge. That is why, you have to have certain enzymes may be, which can break this

type of cellulosic material or you can have mechanical equipment, which produces very high shear so that it can break the tough cellulosic surface of the cell wall. But, if you are culturing plant cells, then they will be less robust and they can be easily broken down. So, cultured plant cells are much more advantageous if you are interested in looking at the intracellular products.

The sizes of animal and plant cells generally vary between 1 to 100 microns. So, you see they are very big. If you go to animal cells, they do not have cell walls. So, that means they are very fragile. So, even cultivating them is a big challenge. If you are going to use mechanical agitator, animal cells can get sheared and broken down. So, generally in one pass in a cell disruptor at about 2000 psi, I can break animal cells.

Whereas if I am looking at blood cells, insect blood cells, they will require much higher pressure. If you are looking at yeast cells, you will require almost 20,000 psi. If you are looking at plant tissue and you want to break it, you are taking about 40,000 psi. So, you see the pressure required to break cells starting from animal to insect blood cells going to yeast cells going to plant tissue, we are talking in the increase of the order of twenty.

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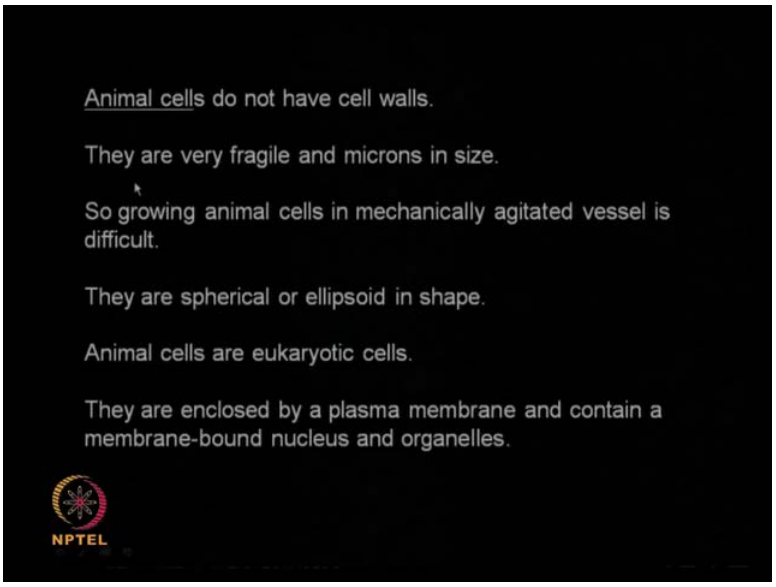

So, animal cells do not have cell walls. So, they are very fragile. They are micron in size. So, as I mentioned for cultivating animal cells in agitated fermentor is a real challenge because they will easily break and form debris. So, you need to use reactors, which do not use mechanical agitation. They are spherical or an ellipsoidal in shape. They are

eukaryotic cells. They are enclosed by a plasma membrane and they contain membrane bound nucleus and organelles.

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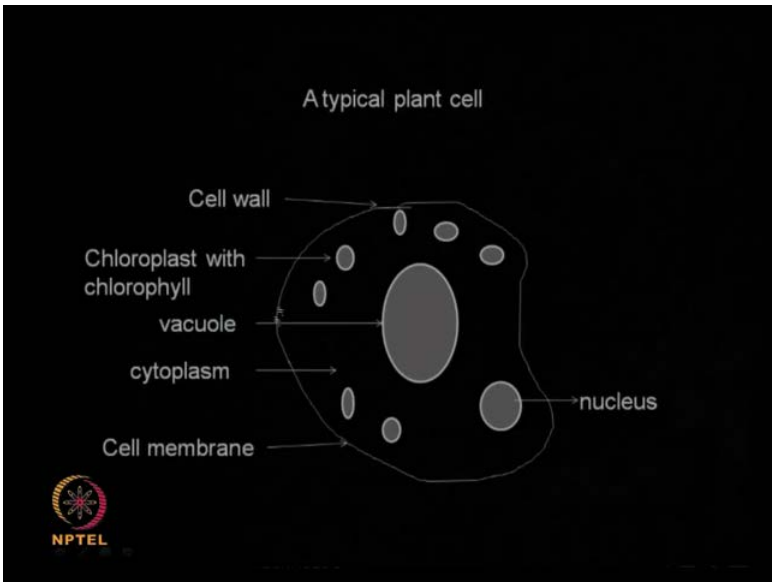

So, typical plant cell is a schematic. You have the cell wall here. You have the chloroplast with the chlorophyll. Then, you have the vacuole, you have the cytoplasm. Here is the cell membrane. Then, you have the nucleus. This is a typical plant cell.

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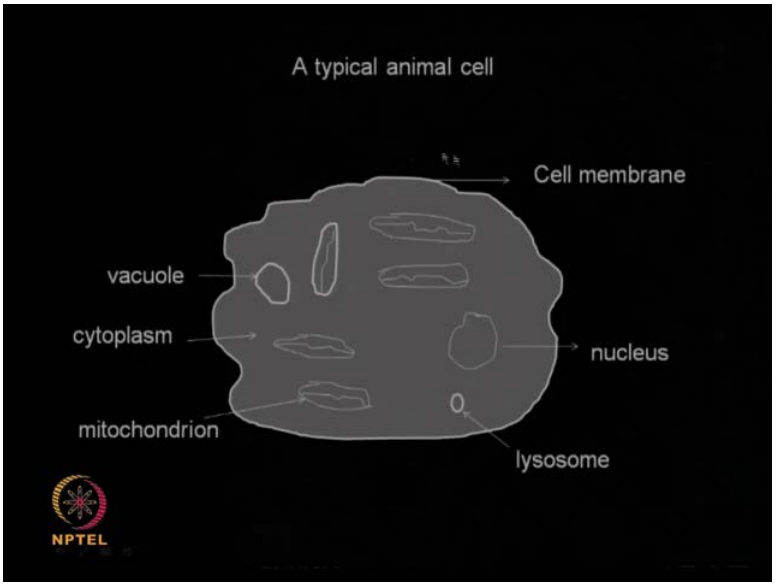

A typical animal cell, you have a very thin cell membrane. Then, you have the nucleus; lysosomes are present here, then mitochondrion, cytoplasm, vacuole. So, you can see a

lot of difference between each one of these cells.

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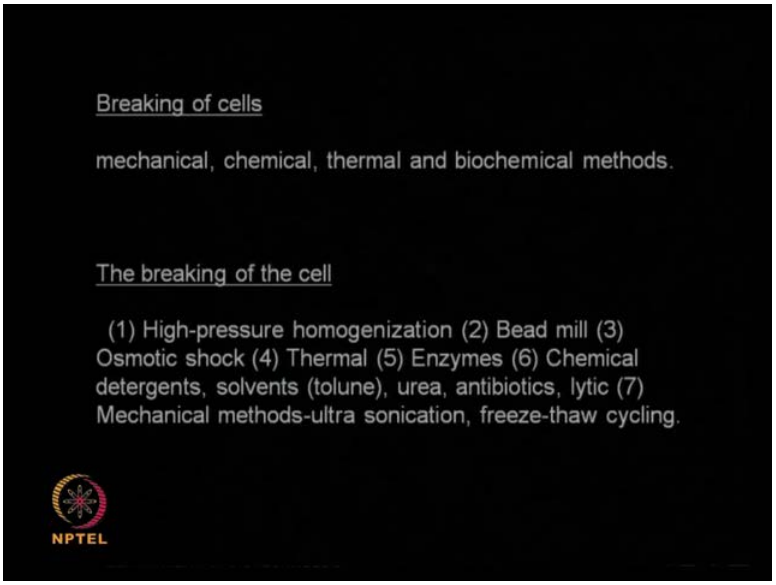

Starting from bacteria the gram positive, gram negative, going down to yeast, going down to molds, going down to animal cells, and finally to the plant cells, very tough business the plant cells. So, there are different ways of breaking the cells. You can resort to mechanical methods. You can resort to chemical methods. You can resort to thermal methods. You can resort to bio chemical methods. When I mean bio chemical, you may be using enzymes to break the cell wall. Each method has its advantages disadvantages, cost components and so on actually.

So, you can select. There is nothing like one single method, which is universally acceptable. You can select the method depending upon all the factors I mentioned in the very second slide. So, for breaking of the cells, you can use a high pressure homogenizer. We will talk about each one of them slightly in detail in subsequent slides, bead mill, you can use osmotic shock, you can use thermal methods, you can use enzymes, and you can use chemical detergents that mean certain anionic or cationic surfactants.

You can use solvents like toluene, you can use urea, and you can use even antibiotics, lytic agents. You can use even ultra sonication, freeze thaw cycling. That means you cool it and then you heat it. You cool it and heat it. So, that is called freeze thaw cycling. So, by doing that, you may be able to break. So, you see, there are so many different mechanical, chemical, thermal and biochemical methods are there and under each

category, you will have several different methods. So, ultimately you may have a choice of about twenty methods available to you. You can select based on the type of organism you are dealing with and type of product quantities you are dealing with.

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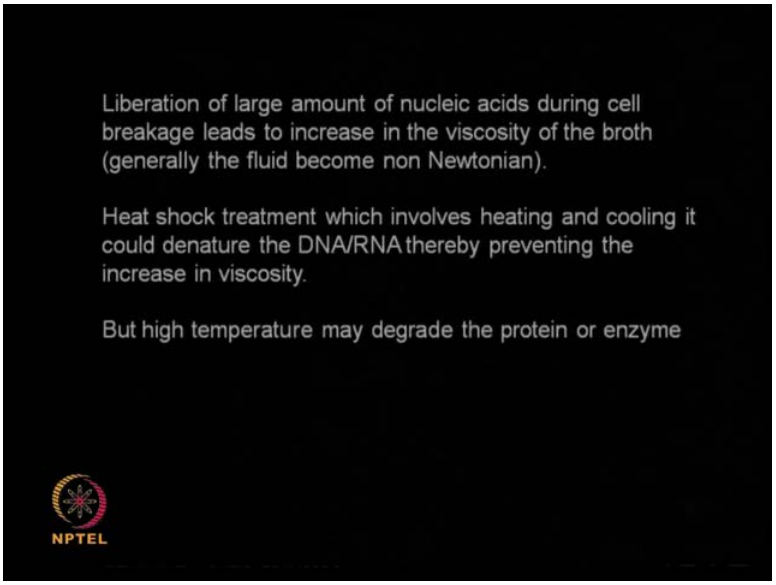

So, when you are breaking the cells, you are liberating a lot of nucleic acids during the cell breakage. So, obviously they increase the viscosity. So, when the viscosity is increased, it is not a Newtonian type of behavior. It becomes non Newtonian. Handling non Newtonian fluid in mechanical agitators, pumping non Newtonian fluid is always a big challenge. So, sometimes you may heat the material and then cool it. That is called the heat shock treatment that will denature your DNA, RNA; thereby you can prevent the viscosity.

It sounds easy, but, then when you are heating it, you may be denaturing your product also. If your product is enzyme or protein, the product itself may be getting denatured. So, you need to be very careful about it. So, you have to understand that the viscosity of the broth after cell breakage is going to rise considerably and it is always a non Newtonian type of behavior.

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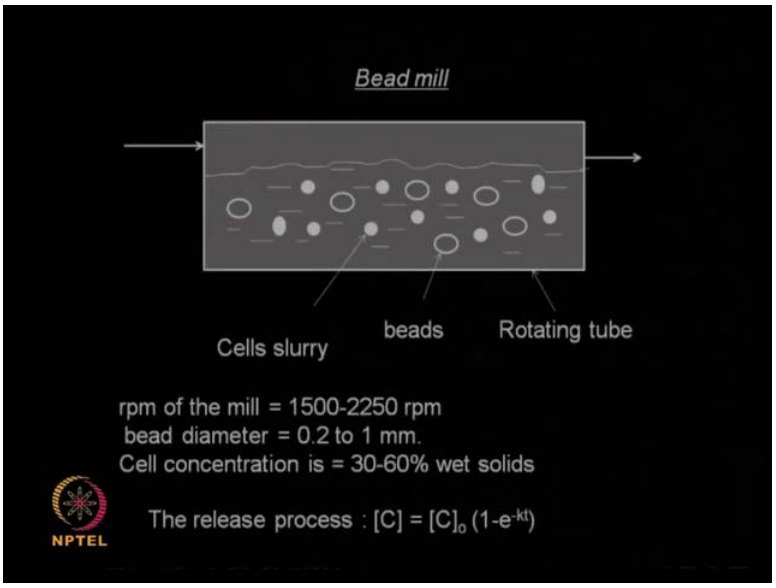

Let us look at various techniques slightly in more detail. This is called a bead mill. As the name implies, it has got metal beads inside, it could be ceramic beads, it could be stainless steel beads, and it could be iron beads. So, you have several beads present inside this. This is cylindrical tubular equipment. It rotates the slurry containing your cells are taken inside. As it rotates, it tumbles and the metal beads hit the cells and because of the mechanical contact, mechanical attrition, mechanical force, the cells break. So, that is the principal of the bead mill.

So, it slowly is revolving around its major axis. This is the major axis. As it revolves, it tumbles, the solid hits the cells and the cells break down. Generally, the rpm is around 1,500 to 2,250 rpm. Bead diameter is about 0.2 to 1 mm size bead.

As I said, the material of construction depends upon the importance of your product, how, whether you can permit contamination or whether you want totally contaminant free operation. So, you may select material according to that. If you take ceramic, ceramic is pretty tough. It can impart a shear to the surface of the microorganism. So, it may be useful for very tough walls. If you want a lot of force, then you may go for metallic type of beads like stainless steel or iron. It can handle cell concentrations of about 30 to 60 percent with solids.

So, it can process quite a lot material, but one main disadvantage of this is after the operation, I have to completely clean because I may have dead cell debris. I may have

exo polysaccharides all stuck inside and that needs to be cleaned so that it does not contaminate the next batch. So, the cleaning operation takes up quite a lot of time. Another disadvantage is temperature. There is a tremendous increase in temperature because this is a mechanical operation and metal beads or ceramic beads hit each other. They also hit the walls of the mill. They also hit the microorganism. So, there is a very high temperature generated inside.

So, if you do not cool the contents, you may denature the proteins or any bio molecules that are produced or that are liberated during this operation. So, if you want to perform two passes of bead mill, then you need to cool after one pass and then the cooled product is again feed into another bead mill. So, the release process is generally we can assume it as a first order process. That means the concentration of the product of interest that is the product, which is released or liberated from the microorganism has a function of time t here is equal to C naught.

This is the total concentration of the product that may be present multiplied by 1 minus exponential power minus k t. k is just like first order; it is a first order rate constant. So, the concentration will slowly rise with the function of time exponentially and it will reach a maximum. Generally, we can assume it as a first order because it is a good approximation to take care and we will do some problems making use of this particular equation.

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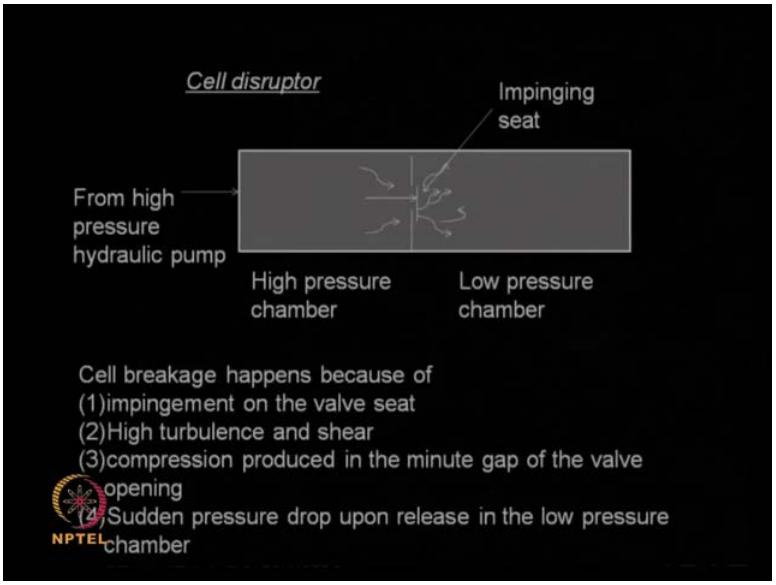

Let us look at another technique by which you could break these cells. This we will call a cell disruptor. So, what you do is you are feeding in the slurry. The slurry means the solution, which contains your cells at a very high pressure using a hydraulic pump. There is a small opening here and there is an impinging seat here and on the other side downstream, the pressure is low. So, here this is a high pressure chamber, this is a low pressure chamber. So, the slurry at very high pressure enters this nozzle, expands, it hits the impinging seat and then it expands into the low pressure region. So, many things happen during this process. That is how the cells break, impingement on the valve seat.

So, the cells hit the valve seat, high turbulence and shear created in this nozzle region, compression produced because the slurry has to enter and flow through the small nozzle and sudden pressure drop. You have high pressure. You have a low pressure so sudden pressure drop. So, all these add up to break the cells that means damage the cells. So, the cell walls break and the intracellular products are released.

This method is slightly better than the bead mill because it does not produce so much heat, but even this also produces heat, but it is not so much as you are the bead mill, which is totally mechanical type of operation. So, here you generate very high pressure. You are talking in terms of 2,000, 4,000 or even 20,000 psi depending upon the type of organism, which you would like to disrupt. So, this is called a cell disruptor.

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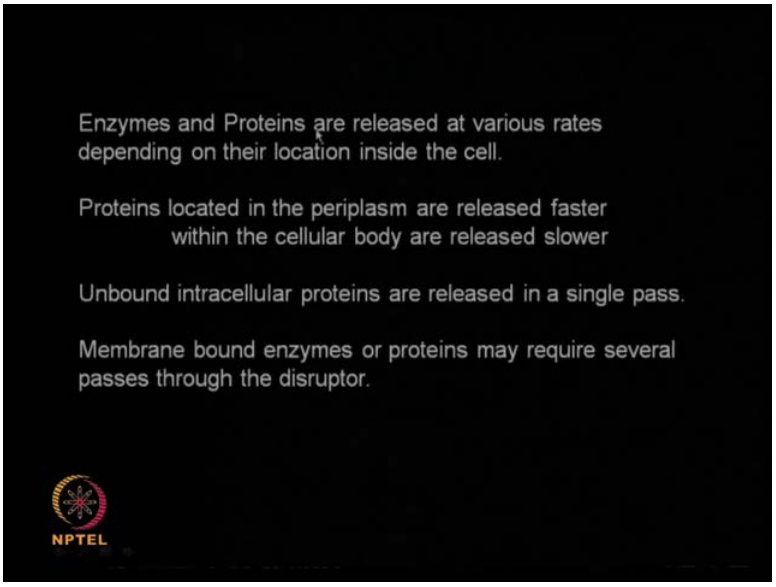

So, enzymes, proteins are released at various rates depending upon where they are

located inside the cell. So if they are located in the periplasmic region, they are released very fast, whereas if they are located inside the cellular body, then they are released slowly. So, you may have fast releasing products, which are coming out of the periplasmic region and you may have slow releasing products, which are coming from the interior of the cell.

So, you should know where your product is located so that you will be able to monitor the product as a function of time. If you have unbound intracellular proteins, then they are released in a single pass. If they are membrane bound enzymes or proteins, then you need to break the membrane so that the membrane bound proteins are released.

So, you need to resort to several passes through the disruptor that means you take out the product. Then, you need to cool the product, again pressurize it, and again pass it through the disruptor and so on. So, you may have to resort to two, three, four, five times through the mechanical disruptor until all the product, which is bound to the membrane is released.

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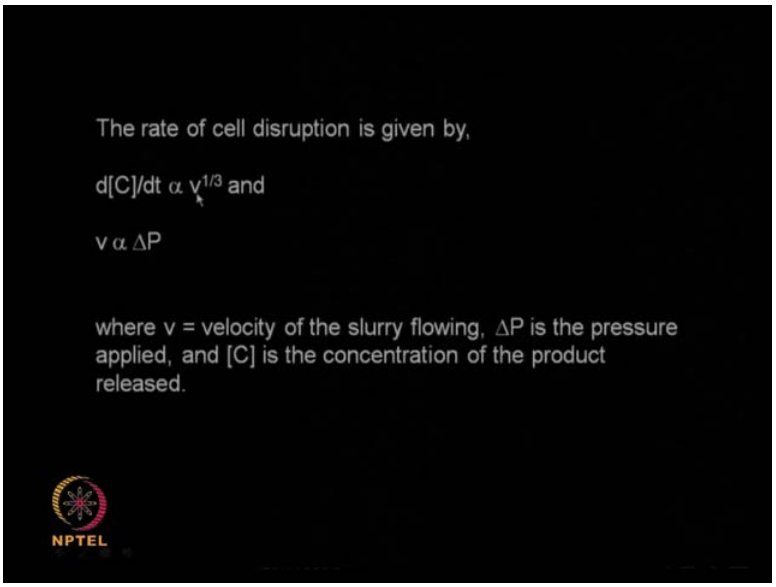

So, the rate of cell disruption rate of cell disruption means d C by d t where C is the concentration, t is the time. D C by d t is proportional to v raise to the power 1 by 3. v is the velocity of the slurry flow and delta P is the pressure, which you are applying. So, it is quite obvious and C is the concentration of the product that is being released actually.

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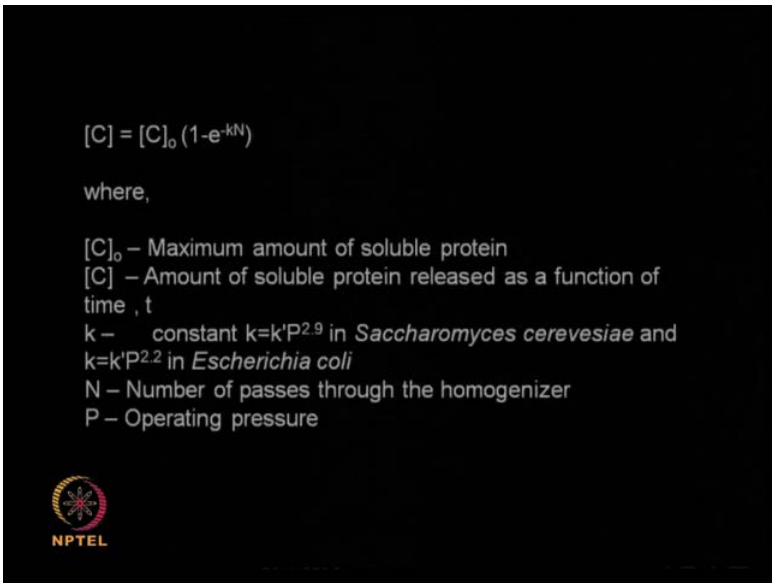

You can reduce this to a much simpler equation of this form C equal to C naught raise to the power 1 minus exponent minus k N. C is the amount of protein or enzyme or product that is released as a function of time t. C naught is the maximum amount of this protein present inside. N is the number of passes through the homogenizer. One pass means N will be one; two passes N will be two and so on.

Now, this k depends upon the type of microorganism and also the operating pressure. So, for example, k equal to k dash P raise to the power 2.9 for saccharomyces or K equal to K dash P raise to the power 2.2 for e coli. So, the exponent varies depending upon the type of microorganism present here actually. So, you see this release depends upon the number of passes, it depends upon the type of microorganisms, and it depends upon the pressure you are applying.

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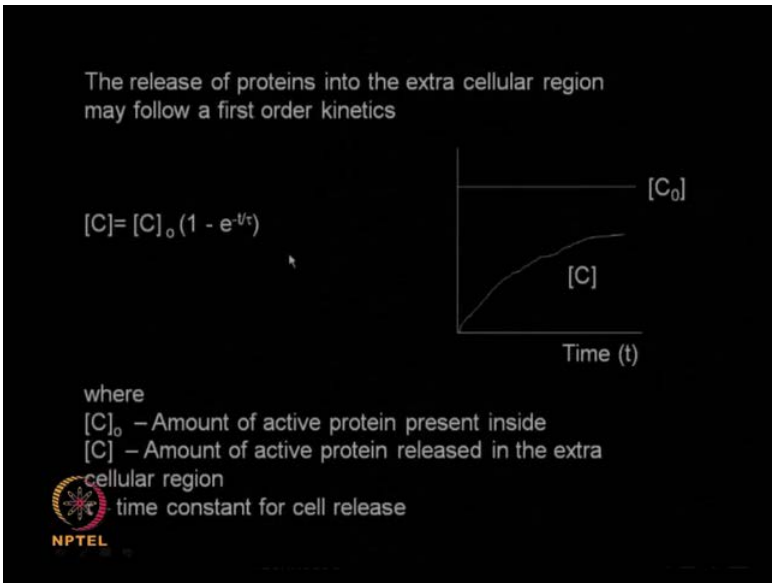

So, obviously this relationship will again be raised, going up exponentially as a function of time. So, we can assume that the proteins are getting released or the intracellular product is coming into the extracellular region as a function of time in a very exponential manner. So, C is equal to C naught 1 minus e minus t by tou. Tou is the time constant for cell release or tou is also equal to related to the rate constant. This is like this is a first order relationship. So, you can have C equal to C naught multiplied by 1 minus e minus k t also because this is a first order relationship.

C naught is the maximum concentration of the product present inside and it is getting released slowly as a function of time. This is assumed as a first order relationship, which is given by this particular equation. If this is a typical first order relationship, tou is called the time constant for the cell release. So, tou will have the same units as t. So, if t is in minutes, tou also will be in minutes. If t is in hours, tou also will be in hours. So, you have to remember that units are the same.

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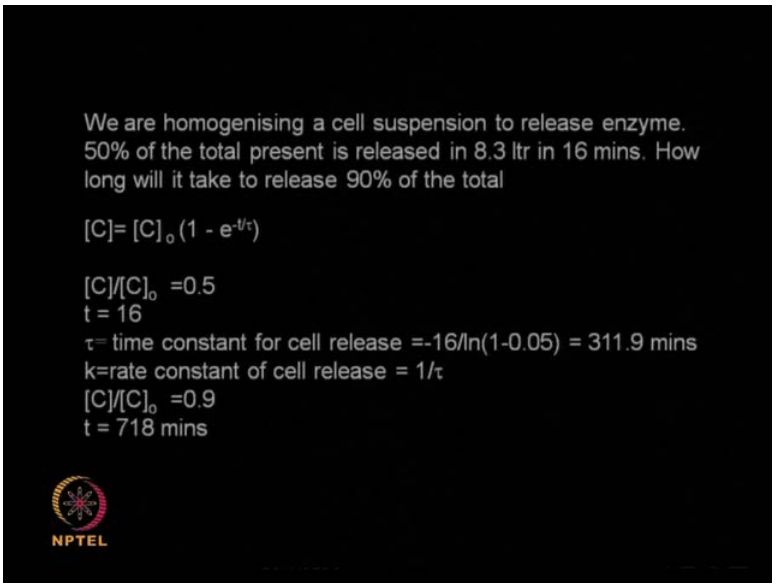

Let us look at a problem here that will explain or that will clarify some of the points, which we talked about related to homogenization as well as the product releases a function of time. So, what we are doing is we are homogenizing a cell suspension to release enzymes present. So, 50 percent of the total present is released in 8.3 liters in 16 minutes. So, how long will it take to release 90 percent of the total amount? This is a very important data to have.

So, if I am having set of cells and I am interested in breaking it and releasing the intracellular product, how long should I wait so that I am sure all the intracellular product is released into the medium? So, in order to do that, I can assume it as a first order relationship, C equal to naught multiplied by 1 minus e raise to the power minus t by tou. As I said, tou is my release constant. C naught is the maximum amount that is present inside the intracellular region. Now, the data that is given is 50 percent of the total present is released in 16 minutes. So, I can put C by C naught as 0.5, I can put t as 16.

So, when I do the calculation here, I will end up calculating time, tou the time constant for cell release as 16 divided by logarithm of 1 minus 0.05 that comes to around 312 minutes. So, this is the time constant for cell release. So, this time constant depends upon the equipment. It depends upon the type of organism I am using, depends upon the pressure I am operating, all those things. So, everything is condensed or encapsulated in

this particular number tou. So, using this, I would like to know how long it will take to release 90 percent of the material.

So, what do I do? I put it back. So, C by C naught will be 0.9 and tou is 311.9. Then, I have to calculate this t. So, t comes out to be 718 minutes. So, you see if I if I am interested in getting 50 percent out, I wait for sixteen minutes, but if I want 90 percent, I need to wait for 718 minutes. So, if I am interested in 95 percent, it may go to about 1000 minutes and if I want 99, it may go to 2,000 or 3,000 minutes. Why is it so? This is because you are assuming a first order type of relationship. So, because it is exponential, it will be slowly moving. As you go further and further, as you reach 90, 92, 95, 98, the time taken will be very, very, very large.

So, it may be easy to collect the sample at smaller quantity, whereas if you are interested in collecting the entire quantity, you may have to wait for very long time and it might not be very economical. So, for example here, 50 percent is collected in 16 minutes, whereas for 90 percent, as you can see, it will require almost 718 minutes. That is a very long time. So, from 16 minutes, it has gone up to 718 minutes. So, if you want to reduce the time, what can we do? We can, either we can increase the pressure or you can modify the equipment. So, you can think of different other methods rather than using the same set up and waiting for a very long time.

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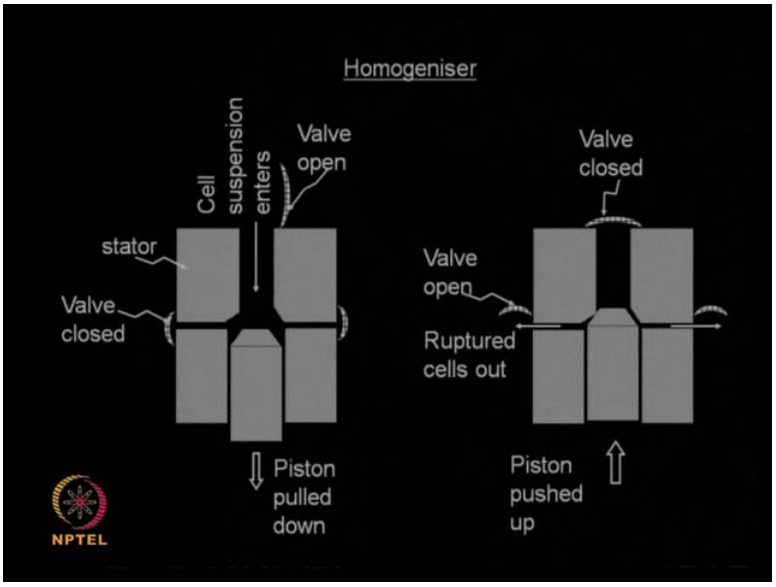

Let us go forward. There is something called homogenizer. So, how does this work? So,

it has got stator here and there is a piston, which moves in and out, in and out. So, when the piston is moving out, it creates a vacuum here, low pressure here. So, the cell suspension comes in. Agreed? This valve opens. So, a cell suspension comes in. When the piston is pushed up, what happens? The slurry is forced through very small hole here and these two valves open. So, they are pushed out at tremendous pressure because you are pushing it out through a small nozzle. So, the cells break. That is what is a homogenizer.

The next time when the piston is pulled down, low pressure is created. These two valves close and these valves open, suspension comes in, so it is almost like your piston in your car. So, in your car, when the piston moves in one direction, petrol comes in. When the piston moves in another direction that petrol gets ignited becomes a gas and so on. So, here when the piston moves down, the cell suspension comes in and then the piston pushes up. So, the slurry is pushed through small nozzle and during the process, the cells break. So, the ruptured cells move out through these nozzles. So, this is called a homogenizer.

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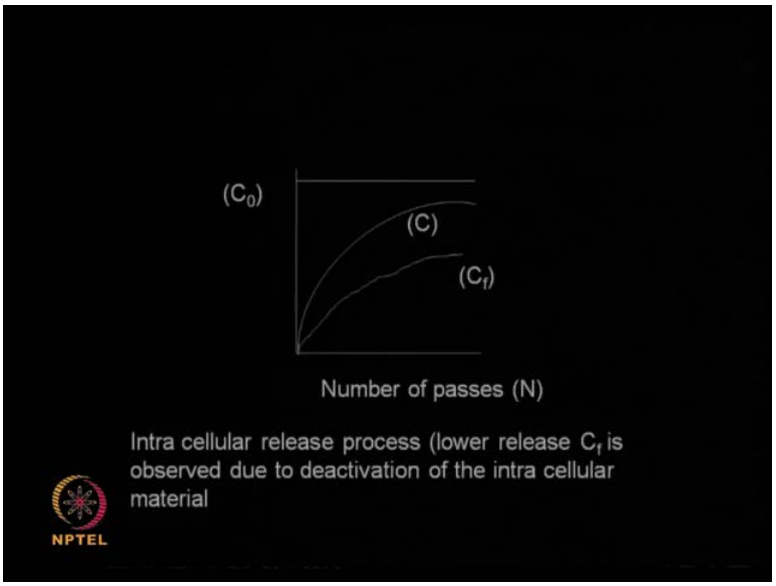

So, just like we had a relationship between C naught, sorry C that is the concentration of the product observed in the extracellular region as a function of time, we can also have a relationship between the concentrations of the product in the extracellular region as a function of number of passes. So, if I keep increasing the number of passes through the

homogenizer or cell disruptor, you are going to collect more material. Generally, it follows a exponential type of relation that is a first type of relation. It is a good approximation to consider.

I have also shown something called Cf. Generally; the concentration of the product in the extracellular region will be slightly lower than what is expected because there could be some deactivation of the product, protein or enzyme because of increase in temperature. This is because as the temperature increases, the enzyme may be getting de activated. So, instead of having a quantity following this particular graph, it may be following this particular graph because there is a denaturation or deactivation of the enzyme taking place because of the rise in temperature inside the mechanical system like a disruptor or homogenizer.

So, our goal is to keep the temperature rise to a minimum so that you do not deactivate your protein, but still, there is going to be some deactivation taking place because of the rise in temperature. So, if you are thinking of many passes, after each pass, the product is externally cooled and then again put back so that the heat that is liberated or generated or accumulated is completely disrupted, otherwise the activity of the enzyme or the protein will go down because of the tremendous amount of heat that is generated inside this mechanical system.

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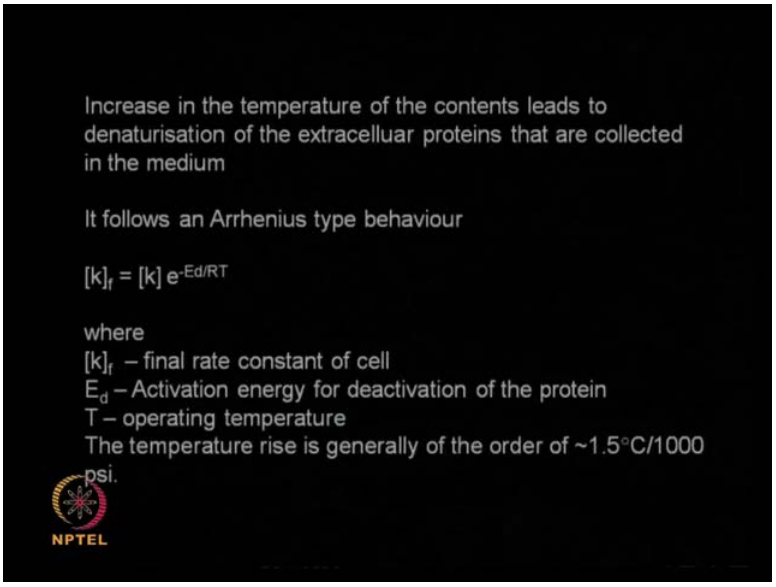

The laws in activity because of the increase in temperature can be also modeled

assuming Arrhenius type of behavior. So, you all must have done may be in first year, the Arrhenius relationship.

So, generally it takes this particular form k that is the rate constant that is the observed rate constant will be equal to the k, theoretical rate constant multiplied by e raise to the power minus Ed by RT. Ed is called the activation energy for deactivation divided by RT. R is your universal gas constant and T is your operating temperature. So, depending upon the operating temperature, this value will be less. If the temperature is high, this value is going to go down. If the temperature is low, this value will be higher and also it will depend upon the deactivation activation energy.

The temperature rise is generally of the order of 1.5 degrees for every 1000 psi. So, if the pressure increases for every 1000 psi, there will be the temperature rise inside your homogenizer or any mechanical instrument equipment will be about 1.5 degree centigrade. So, we can use this particular equation to calculate what will be the apparent rate constant of release of the product if the product gets deactivated because of the rise in temperature. Understand?

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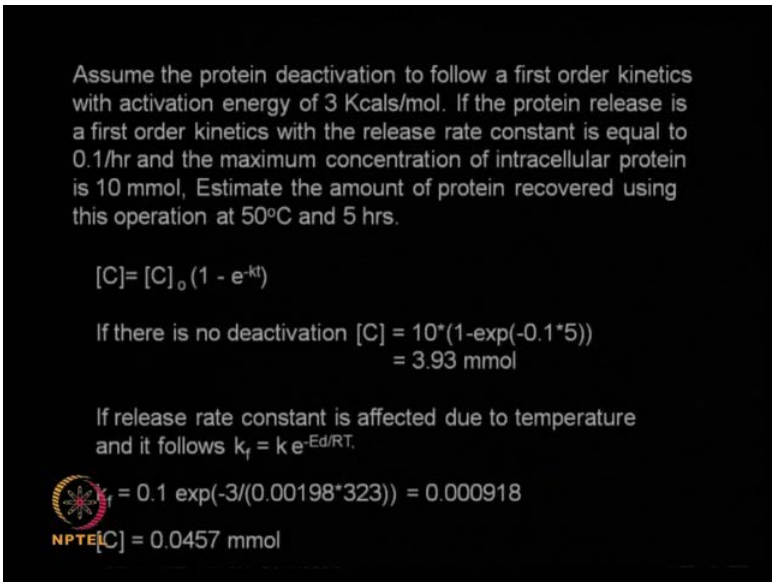

For example, let us look at this problem. This combines both the aspects, the release of the intracellular product; it follows a first order kinetics, the deactivation of the product because of the increase in temperature that follows Arrhenius behavior. So, I can combine both these and tell what will be the actual concentration of the desired product.

Let us look at this problem. Assume a protein deactivating and it follows a first order kinetics with activation energy of 3 kilo cals per mole. Now, this protein release is the first order kinetics. If the release rate constant is equal to 0.1 per hour and the maximum concentration of the intracellular protein is 10millimole. Estimate the amount of protein recovered using this operation at 50 degree centigrade and 5 hours. That means after 5 hours, how much protein you will collect if you are doing it at 50 degree centigrade?

So, the protein release is following a first order kinetics. It is given here. So, we talked about this equation quite a lot, C is equal to C naught multiplied by 1 minus e raise to the power minus kt. Now, C naught is 10millimole. Now, your k if there is no deactivation will be 0.1 per hour and time is 5 hours. So, I can put in all these and I get it as 3.93. That means at the end of 5 hours, if there is no deactivation taking place, I just put the numbers here and show that I will be collecting 3.93millimoles of the product or the protein.

Now, if the release rate is also affected because of deactivation because of the increase in temperature, then let us consider an Arrhenius type of relationship. As I showed in the previous slide, K f is equal to K into e power minus Ed by RT. Ed is your deactivation activation energy, which is given as 3 kilo cals per mole, R is equal to 1.98 calories per gram mole per Kelvin. I hope you all know that. So, I have put 0.001 here. Temperature is 50 degree centigrade. So, 273 plus 50 degree is 323 K. In the absence of deactivation, the k value is 0.1. So, I can put in all these data inside and calculate my K f. So, you see that comes out very small.

If I substitute that K here for 5 hours, I will get product release as 0.0457millimole. So, if there is no deactivation at the end of 5 hours, I will be collecting 3.93millimole of the protein. If I assume a deactivation, if I assume a constant rate of deactivation and it behaves in this fashion that means an Arrhenius type of behavior, then the amount of protein I will be collecting at the end of the 5 hours will be very small.

You can see the difference because of the deactivation of the protein because of the temperature here. So, you see that temperature will play a very important role in the amount of product you are going to collect at the end of the process. That is why many of the mechanical agitators may have inline cooling that means that there may be cooling facility inside the equipment itself so that the temperature rise is practically minimal;

may be 1 or 2 degrees, not in terms of large values.

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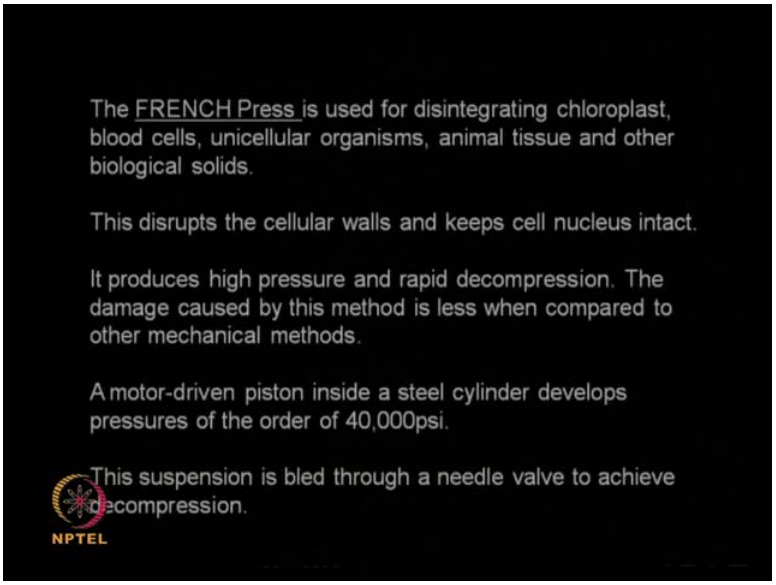

The next mechanical based cell breakage is called the French press. The French press is used for a disintegrating chloroplast. It can be used for blood cells; it can be used for unicellular organisms, animal tissues and several other biological solids. The interesting thing about French press is it will just disrupt the cellular walls and it will keep the nucleus intact. So, how it works is it produces a very high pressure and rapid decompression.

So, there is not much damage caused by this method unlike the bead mill or any other type of a mechanical method actually. So, what you do is you produce very; very high pressure and you suddenly reduce the pressure. That is how it works actually. So, you are talking in terms of almost 40,000 psi.

So, your motor driven piston inside a steel cylinder develops this type of pressure. Then, this high pressure slurry is bled through a needle valve. So, when you bleed it through a needle valve, you are achieving a very fast decompression. During this process, the cells break, but the nucleus remains intact. The temperature rise is not as dramatic as in the other mechanical methods, which I talked about actually, but basically, this also involves high pressure and a low pressure operation. So, we talked about different types of mechanical operations, which can help you to disrupt your cells or break your cell walls and release the contents.

Then, during the process, I said increase in temperature is a very important point to note. When the temperature rises if you assume a deactivation of a bio molecule based on Arrhenius type of behavior; then the rate constant of release gets affected because of the deactivation. So, the amount of product that gets released during this operation will be much less because of the increase in temperature, which in turn affects your rate constant, which in turn affects your product release rate. So, you have to decide on which type of mechanical technique you want to resort to if you want to break cells and whether the product can withstand this increase in temperature.

When compared to the bead mill or when you compare to homogenizer, French press is better because the amount of temperature rise you will observe in this equipment is much less when compared to the equipment like bead mill. Bead mill also requires more cleaning after the process. So, it consumes the overall batch cycle time is also much larger when compared to some other technique. So, now mechanical operations are although problematic, they are quite cheap. They are quite robust and easy to handle.

That is why in large scale operations that means during scale up, many groups prefer mechanical based cell disruption methods rather than chemical or enzymatic methods because they are quite cheap. They can be easily scalable, they can be easily handled and the ease of operation is also much better when compared to higher techniques.